

At page 121, line 12, please delete "C (asp)" all three occurrences and substitute therefor --C (ala)--.

At page 122, line 11, please delete "C (asp)" all three occurrences and substitute therefor --C (ala)--.

IN THE CLAIMS:

Please cancel claims 1-62 without prejudice.

Please add new claims 63-163 as follows consistent with the restriction requirement mailed on December 10, 1997 in the above identified parent application.

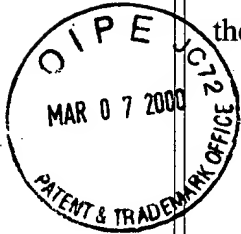
1 --63. An isolated infectious recombinant respiratory syncytial virus (RSV)
2 comprising a RSV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid
3 phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase elongation factor,
4 wherein a modification is introduced within the genome or antigenome comprising a partial or
5 complete gene deletion, a change in gene position, or one or more nucleotide change(s) that
6 modulate expression of a selected gene.

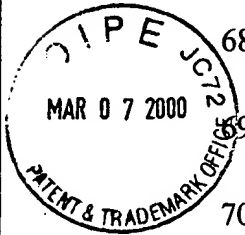
1 64. The recombinant RSV of claim 63, wherein said gene is selected from
2 an attachment (G) protein, fusion (F) protein, small hydrophobic (SH) protein, RNA binding
3 protein (N), phosphoprotein (P), large polymerase protein (L), M2(ORF1) or M2(ORF2)
4 product, matrix (M) protein, or a nonstructural protein NS1 or NS2.

1 65. The recombinant RSV of claim 63, wherein a RSV gene is deleted in
2 whole or in part.

1 66. The recombinant RSV of claim 65, wherein a SH, NS1, NS2, or G gene
2 is deleted in whole or in part.

1 67. The recombinant RSV of claim 66, wherein the SH gene is deleted.





- 1 68. The recombinant RSV of claim 66, wherein the NS1 gene is deleted.
- 1 69. The recombinant RSV of claim 66, wherein the NS2 gene is deleted.
- 1 70. The recombinant RSV of claim 63, wherein expression of a selected
2 RSV gene is reduced or ablated by introduction of one or more translation termination codons.
- 1 71. The recombinant RSV of claim 70, wherein expression of a selected
2 RSV gene is reduced or ablated by introduction of multiple translation termination codons.
- 1 72. The recombinant RSV of claim 71, wherein expression the RSV NS2
2 gene is reduced or ablated by introduction of multiple translation termination codons
- 1 73. The recombinant RSV of claim 63, wherein expression of a selected
2 RSV gene is reduced or ablated by introduction of a frame shift mutation in the gene.
- 1 74. The recombinant RSV of claim 63, wherein expression of a selected
2 RSV gene is modulated by introduction, modification or ablation of a translational start site
3 within the gene.
- 1 75. The recombinant RSV of claim 74, wherein a translational start site of
2 the selected gene is modified or ablated to prevent efficient translation initiation at said start
3 site.
- 1 76. The recombinant RSV of claim 74, wherein an internal translational start
2 site of the selected gene is modified or ablated to prevent efficient translation initiation at said
3 start site.
- 1 77. The recombinant RSV of claim 74, wherein an internal translational start
2 site of the RSV G gene is ablated to prevent efficient translation initiation at said start site
3 specifying expression of a secreted form of the G protein.
- 1 78. The recombinant RSV of claim 74, wherein a translational start site is
2 introduced upstream of the selected gene or internally to enhance expression of the gene.

1 79. The recombinant RSV of claim 63, wherein a position of one or more
2 gene(s) in the genome or antigenome is altered relative to a RSV promoter.

1 80. The recombinant RSV of claim 79, wherein a position of said one or
2 more gene(s) is changed to a more promoter-proximal location specifying enhanced expression
3 of the gene(s).

1 81. The recombinant RSV of claim 80, wherein said position of said one or
2 more gene(s) is changed to a more promoter-proximal location by deletion of coding or non-
3 coding sequences within the genome or antigenome upstream of said one or more gene(s).

1 82. The recombinant RSV of claim 81, wherein positions of multiple RSV
2 gene(s) are changed to a more promoter-proximal location by deletion of a SH or NS2 gene or
3 genome segment.

1 83. The recombinant RSV of claim 79, wherein a position of said one or
2 more gene(s) is changed to a more promoter-distal location specifying reduced expression of
3 the gene(s).

1 84. The recombinant RSV of claim 81, wherein a coding or non-coding
2 polynucleotide sequence selected from an autologous or heterologous RSV or non-RSV gene
3 or gene segment is inserted in the genome or antigenome upstream of said one or more gene(s).

1 85. The recombinant RSV of claim 79, wherein positions of multiple genes
2 in the genome or antigenome are altered by changing their relative gene order.

1 86. The recombinant RSV of claim 85, wherein the positions of multiple
2 genes are altered by reciprocal positional substitution of said genes in the genome or
3 antigenome.

1 87. The RSV of claim 86, wherein the NS2 gene is reciprocally substituted
2 in position for the SH gene.

1 88. The recombinant RSV of claim 63, wherein said modification within the
2 genome or antigenome comprising a partial or complete gene deletion, a change in gene
3 position, or one or more nucleotide change(s) that modulate expression of a selected gene
4 specifies a change in phenotype for the resultant recombinant virus selected from a change in
5 growth characteristics in culture, small plaque size, attenuation in vivo, temperature-sensitivity,
6 cold-adaptation, host range restriction, change in antigen expression, or a change in
7 immunogenicity.

1 89. The recombinant RSV of claim 63, wherein the genome or antigenome
2 is further modified to incorporate one or more attenuating mutation(s) present in one or more
3 biologically derived mutant human RSV strain(s).

1 90. The recombinant RSV of claim 89, wherein the genome or antigenome
2 is further modified to incorporate at least one and up to a full complement of attenuating
3 mutations present within a panel of biologically derived mutant human RSV strains, said panel
4 comprising cpts RSV 248 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV
5 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC VR
6 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542), and RSV
7 B-1 cp-23 (ATCC VR 2579).

1 91. The recombinant RSV of claim 89, wherein the genome or antigenome
2 is further modified to incorporate at least one and up to a full complement of attenuating
3 mutations specifying an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or
4 Thr523 in the RSV F gene, Cys319, Phe 521, Gln831, Met1169, Tyr1321 and/or His 1690 in
5 the RSV polymerase gene L, and a nucleotide substitution in the gene-start sequence of gene
6 M2.

1 92. The recombinant RSV of claim 89, wherein the genome or antigenome
2 is further modified to incorporate at least one mutation specifying a temperature-sensitive
3 substitution at amino acid Phe521, Gln831, Met1169, or Tyr1321 in the RSV polymerase gene
4 or a temperature- sensitive nucleotide substitution in the gene-start sequence of gene M2.

1 93. The recombinant RSV of claim 89, wherein the genome or antigenome
2 incorporates at least two attenuating mutations.

1 94. The RSV of claim 1, having at least three attenuating mutations.

1 95. The recombinant RSV of claim 89, wherein the genome or antigenome
2 includes at least one attenuating mutation stabilized by multiple nucleotide changes in a codon
3 specifying the mutation.

1 96. The recombinant RSV of claim 63, wherein the genome or antigenome
2 comprises a partial or complete human RSV genome or antigenome of one RSV subgroup or
3 strain combined with a heterologous gene or gene segment from a different, human or non-
4 human RSV subgroup or strain to form a chimeric genome or antigenome.

1 97. The recombinant RSV of claim 96, wherein the heterologous gene or
2 gene segment is from a human RSV subgroup A, human RSV subgroup B, bovine RSV, or
3 murine RSV.

1 98. The recombinant RSV of claim 96, wherein the heterologous gene or
2 gene segment is selected from a RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F or
3 G gene or gene segment.

1 99. The recombinant RSV of claim 96, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV A subgroup genome or antigenome
3 combined with a heterologous gene or gene segment from a human RSV B subgroup virus.

1 100. The recombinant RSV of claim 99, wherein the heterologous gene or
2 gene segment from human RSV B encodes a RSV F, G or SH glycoprotein or a cytoplasmic
3 domain, transmembrane domain, ectodomain or immunogenic epitope thereof.

1 101. The recombinant RSV of claim 100, wherein one or more human RSV B
2 subgroup glycoprotein genes F, G and SH or a cytoplasmic domain, transmembrane domain,
3 ectodomain or immunogenic epitope thereof is substituted within a partial RSV A genome or
4 antigenome.

1 102. The recombinant RSV of claim 101, wherein both human RSV B
2 subgroup glycoprotein genes F and G are substituted to replace counterpart F and G
3 glycoprotein genes in the RSV A genome or antigenome.

1 103. The recombinant RSV of claim 96, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV B subgroup genome or antigenome
3 combined with a heterologous gene or gene segment from a human RSV A subgroup virus.

1 104. The recombinant RSV of claim 63, wherein the chimeric genome or
2 antigenome comprises a partial or complete RSV background genome or antigenome of a
3 human or bovine RSV combined with a heterologous gene or genome segment of a different
4 RSV to form a human-bovine chimeric RSV genome or antigenome.

1 105. The recombinant RSV of claim 104, wherein the heterologous gene or
2 genome segment is substituted for a counterpart gene or genome segment in a partial RSV
3 background genome or antigenome.

1 106. The recombinant RSV of claim 104, wherein the heterologous gene or
2 genome segment is added adjacent to or within a noncoding region of the partial or complete
3 RSV background genome or antigenome.

1 107. The recombinant RSV of claim 104, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV background genome or antigenome
3 combined with a heterologous gene or genome segment from a bovine RSV.

1 108. The recombinant RSV of claim 63, wherein the genome or antigenome
2 is further modified to incorporate a nucleotide deletion, insertion, substitution, rearrangement,
3 or modification of a cis-acting regulatory sequence within the recombinant RSV genome or
4 antigenome.

1 109. The recombinant RSV of claim 108, wherein the cis-acting regulatory
2 sequence occurs within a 3' leader, 5' trailer or intergenic region of the RSV genome or
3 antigenome.

1 110. The recombinant RSV of claim 108, wherein the cis-acting regulatory
2 sequence is a gene-start (GS) signal, a (GE) signal, or a RSV promoter element.

1 111. The recombinant RSV of claim 108, wherein the cis-acting regulatory
2 sequence is a gene-start (GS) or gene-end (GE) signal which is modified, deleted, inserted or is
3 replaced by a heterologous GS or GE signal in the genome or antigenome.

1 112. The recombinant RSV of claim 111, wherein a GE signal of the RSV
2 NS1 or NS2 gene is replaced by a corresponding GE signal of the RSV N gene.

1 113. The recombinant RSV of claim 108, wherein the cis-acting regulatory
2 sequence is replaced by a heterologous regulatory sequence.

1 114. The recombinant RSV of claim 113, wherein the heterologous
2 regulatory sequence is a cis-acting regulatory sequence of a different RSV gene.

1 115. The recombinant RSV of claim 108, wherein a RSV promoter element is
2 replaced by a heterologous promoter from a different RSV.

1 116. The recombinant RSV of claim 63, wherein the genome or antigenome
2 incorporates a heterologous gene or genome segment from parainfluenza virus (PIV).

1 117. The recombinant RSV of claim 116, wherein the gene or genome
2 segment encodes a PIV HN or F glycoprotein or immunogenic domain or epitope thereof.

1 118. The recombinant RSV of claim 116, wherein the genome segment
2 encodes one or more immunogenic protein(s), protein domain(s) or epitope(s) HPIV1, HPIV2,
3 and/or HPIV3.

1 119. The recombinant RSV of claim 63, wherein the genome or antigenome
2 is further modified to encode a non-RSV molecule selected from a cytokine, a T-helper
3 epitope, or a protein of a microbial pathogen capable of eliciting a protective immune response
4 in a mammalian host.

- 1 120. The recombinant RSV of claim 63 which is a virus.
- 1 121. The recombinant RSV of claim 63 which is a subviral particle.
- 1 122. The recombinant RSV of claim 63, formulated in a dose of 10³ to 10⁶
2 PFU of attenuated virus.
- 1 123. A method for stimulating the immune system of an individual to induce
2 protection against respiratory syncytial virus, which comprises administering to the individual
3 an immunologically sufficient amount of the recombinant RSV of claim 63.
- 1 124. The method of claim 123, wherein the recombinant virus is administered
2 in a dose of 10³ to 10⁶ PFU of the attenuated RSV.
- 1 125. The method of claim 123, wherein the recombinant virus is administered
2 to the upper respiratory tract.
- 1 126. The method of claim 125, wherein the recombinant virus is administered
2 by spray, droplet or aerosol.
- 1 127. The method of claim 123, wherein the recombinant virus is administered
2 to an individual seronegative for antibodies to RSV or possessing transplacentally acquired
3 maternal antibodies to RSV.
- 1 128. A vaccine to induce protection against RSV, which comprises an
2 immunologically sufficient amount of the recombinant RSV of claim 63 in a physiologically
3 acceptable carrier.
- 1 129. The vaccine of claim 128, formulated in a dose of 10³ to 10⁶ PFU of the
2 attenuated RSV.
- 1 130. The vaccine of claim 128, formulated for administration to the upper
2 respiratory tract by spray, droplet or aerosol.

1 131. The vaccine of claim 128, wherein the recombinant RSV elicits an
2 immune response against human RSV A, human RSV B, or both.

1 132. An expression vector comprising an isolated polynucleotide molecule
2 encoding a respiratory syncytial virus (RSV) genome or antigenome modified by a partial or
3 complete gene deletion, a change in gene position, or one or more nucleotide change(s) that
4 modulate expression of a selected gene.

1 133. An isolated polynucleotide molecule comprising a respiratory syncytial
2 virus (RSV) genome or antigenome which is modified by a partial or complete gene deletion, a
3 change in gene position, or one or more nucleotide change(s) that modulate expression of a
4 selected gene.

1 134. The isolated polynucleotide molecule of claim 133, wherein a RSV gene
2 is deleted in whole or in part.

1 135. The isolated polynucleotide molecule of claim 134, wherein a SH, NS1,
2 NS2, or G gene is deleted in whole or in part.

1 136. The isolated polynucleotide molecule of claim 135, wherein the SH gene
2 is deleted.

1 137. The isolated polynucleotide molecule of claim 135, wherein the NS1
2 gene is deleted.

1 138. The isolated polynucleotide molecule of claim 135, wherein the NS2
2 gene is deleted.

1 139. The isolated polynucleotide molecule of claim 133, wherein expression
2 of a selected RSV gene is reduced or ablated by introduction of one or more translation
3 termination codons.

1 140. The isolated polynucleotide molecule of claim 133, wherein expression
2 of a selected RSV gene is reduced or ablated by introduction of a frame shift mutation in the
3 gene.

1 141. The isolated polynucleotide molecule of claim 133, wherein expression
2 of a selected RSV gene is modulated by introduction, modification or ablation of a
3 translational start site within the gene.

1 142. The isolated polynucleotide molecule of claim 141, wherein a
2 translational start site of the selected gene is modified or ablated to prevent efficient translation
3 initiation at said start site.

1 143. The isolated polynucleotide molecule of claim 141, wherein an internal
2 translational start site of the selected gene is modified or ablated to prevent efficient translation
3 initiation at said start site.

1 144. The isolated polynucleotide molecule of claim 143, wherein an internal
2 translational start site of the RSV G gene is ablated to prevent efficient translation initiation at
3 said start site specifying expression of a secreted form of the G protein.

1 145. The isolated polynucleotide molecule of claim 141, wherein a
2 translational start site is introduced upstream of the selected gene or internally to enhance
3 expression of the gene.

1 146. The isolated polynucleotide molecule of claim 133, wherein a position
2 of one or more gene(s) in the genome or antigenome is altered relative to a RSV promoter.

1 147. The isolated polynucleotide molecule of claim 133, wherein said
2 modification within the genome or antigenome comprising a partial or complete gene deletion,
3 a change in gene position, or one or more nucleotide change(s) that modulate expression of a
4 selected gene specifies a change in phenotype for the resultant recombinant virus selected
5 from: a change in growth characteristics in culture, small plaque size, attenuation in vivo,

6 temperature-sensitivity, cold-adaptation, host range restriction, change in antigen expression,
7 or a change in immunogenicity.

1 148. The isolated polynucleotide molecule of claim 133, wherein the genome
2 or antigenome is further modified to incorporate one or more attenuating mutation(s) present in
3 one or more biologically derived mutant human RSV strain(s).

1 149. The isolated polynucleotide molecule of claim 148, wherein the genome
2 or antigenome is further modified to incorporate at least one and up to a full complement of
3 attenuating mutations specifying an amino acid substitution at Val267 in the RSV N gene,
4 Glu218 and/or Thr523 in the RSV F gene, Cys319, Phe 521, Gln831, Met1169, Tyr1321
5 and/or His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start
6 sequence of gene M2.

1 150. The isolated polynucleotide molecule of claim 148, wherein the genome
2 or antigenome incorporates at least two attenuating mutations.

1 151. The isolated polynucleotide molecule of claim 133, wherein the genome
2 or antigenome comprises a partial or complete human RSV genome or antigenome of one RSV
3 subgroup or strain combined with a heterologous gene or gene segment from a different,
4 human or non-human RSV subgroup or strain to form a chimeric genome or antigenome.

1 152. The isolated polynucleotide molecule of claim 151, wherein the
2 heterologous gene or gene segment is from a human RSV subgroup A, human RSV subgroup
3 B, bovine RSV, or murine RSV.

1 153. The isolated polynucleotide molecule of claim 152, wherein the
2 chimeric genome or antigenome comprises a partial or complete human RSV A subgroup
3 genome or antigenome combined with a heterologous gene or gene segment from a human
4 RSV B subgroup virus.

1 154. The isolated polynucleotide molecule of claim 63, wherein the chimeric
2 genome or antigenome comprises a partial or complete RSV background genome or

antigenome of a human or bovine RSV combined with a heterologous gene or genome segment of a different RSV to form a human-bovine chimeric RSV genome or antigenome.

155. The isolated polynucleotide molecule of claim 154, wherein the chimeric genome or antigenome comprises a partial or complete human RSV background genome or antigenome combined with a heterologous gene or genome segment from a bovine RSV.

156. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome is further modified to incorporate a nucleotide deletion, insertion, substitution, rearrangement, or modification of a cis-acting regulatory sequence within the recombinant RSV genome or antigenome.

157. The isolated polynucleotide molecule of claim 156, wherein the cis-acting regulatory sequence is a gene-start (GS) signal, a gene-end (GE) signal, or a RSV promoter element.

158. The isolated polynucleotide molecule of claim 157, wherein the cis-acting regulatory sequence is a gene-start (GS) or gene-end (GE) signal which is modified, deleted, inserted or is replaced by a heterologous GS or GE signal in the genome or antigenome.

159. The isolated polynucleotide molecule of claim 158, wherein a GE signal of the RSV NS1 or NS2 gene is replaced by a corresponding GE signal of the RSV N gene.

160. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome incorporates a heterologous gene or genome segment from parainfluenza virus (PIV).

161. The isolated polynucleotide molecule of claim 133, wherein the genome or antigenome is further modified to encode a non-RSV molecule selected from a cytokine, a T-helper epitope, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.